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Activity-Dependent Synaptogenesis in the Adult Mammalian Cortex

Minireview

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Recent electron microscopic studies provide evidence that the adult cortex generates new synapses in response to sensory activity and that these structural changes can occur rapidly, within 24 hr of sensory stimulation. Together with progress imaging synapses in vivo, the stage appears set for advances in understanding the dynamics and mechanisms of experience-dependent synaptogenesis.

One of the central problems of neurobiology is how neural circuits form and how their structure and function are regulated. The pattern of synaptic connections supports all aspects of brain function, from sensory perception and movement to learning and memory. Despite considerable progress in identifying the molecular constituents of central synapses and increasing information on the time course of their assembly, many key questions about synapse formation remain. What are the signals that initiate synaptogenesis? What determines the rate and placement of new synapses? How is synaptic growth influenced by sensory experience?

Formation of synapses during development is thought to depend upon both genetics and environmental influences. Initial establishment of synaptic connections occurs independent of experience, followed by a period of experience-dependent refinement. A common view held that, after this critical period of fine-tuning, the resulting configuration of synaptic connections remained unaltered throughout the lifetime of the animal. However, research over the last decade has provided evidence for extensive experience-dependent plasticity in the adult brain (Gilbert, 1998).

Some of the most detailed mechanistic studies of experience-dependent plasticity have been performed in the rodent barrel cortex. In layer IV of this somatosensory cortex, neurons are arranged in discrete clusters called "barrels." Each whisker activates neurons primarily in a single identifiable barrel, and the spatial arrangement is topographic, such that neighboring whiskers map to neighboring barrels. Manipulations of the sensory periphery during development and in the adult, such as through clipping whiskers, can change the receptive fields of cortical neurons. Robust experience-dependent plasticity can be measured as soon as 1 day after whisker clipping (e.g., Diamond et al., 1993). Most models hold that the cellular basis of this experience-dependent plasticity is due to modifications of existing synapses, such as long-term potentiation and depression (Buonomano and Merzenich, 1998).

A different view has taken center stage in studies of learning and memory. Although short-term changes in synaptic strength are attributed to changes in existing synapses, it has been proposed that long-term changes in synaptic strength are accompanied by structural rearrangements, through formation or elimination of synapses (Bailey and Kandel, 1993). In the adult cerebral cortex, support for structural dynamics, including synapse formation and elimination, has come in response to prolonged alterations of sensory experience (e.g., Darian-Smith and Gilbert, 1994; Kleim et al., 1996). There was little evidence for rapid synaptogenesis in the adult animal in response to sensory stimulation, until the publication of a recent paper in *Neuron* (Knott et al., 2002). This study provides clear evidence that synaptogenesis occurs rapidly in the barrel cortex of adult mice in response to whisker stimulation.

Ultrastructural Evidence for Activity-Dependent Synaptogenesis in the Adult Cortex

Knott et al. studied effects of stimulating a single whisker on synapses in the barrel cortex of mice (Knott et al., 2002). They attached a small metal filing to a whisker and then placed the animal into a cage contained in the core of an electromagnet. Using an oscillating magnetic field, they were able to move the iron filing and thereby deflect the whisker. Sensory stimuli consisted of 5 Hz deflections for 24 hr. This method allowed them to stimulate in the freely moving animal. Immediately after stimulation, they analyzed the layer IV neuropil, using serial section electron microscopy. Remarkably, after this relatively brief period of stimulation, they observed a 35% increase in synapse density specifically in the barrel corresponding to the stimulated whisker.

Most excitatory synapses in the cortex occur on dendritic spines, tiny protrusions emanating from dendrites. In developing pyramidal neurons in vitro (Dailey and Smith, 1996) and in vivo (Lendvai et al., 2000) spines show extensive motility, suggesting a role for spine dynamics in synapse formation and plasticity. The observed 35% increase in synapse density was accompanied by a 25% increase in spine density and a 3.5-fold increase in the density of spines with two synapses. These data suggest that the new synapses form predominantly on spines, either through addition of a second synapse on a preexisting spine or by growth of new spines.

Immunohistochemistry for GABA revealed that there was a similar increase in density of inhibitory (0.09 μm^{-3}) and excitatory (0.10 μm^{-3}) synapses after 24 hr of whisker stimulation. However, because there are many more excitatory than inhibitory synapses in the cortex, the proportional increase in inhibition (2-fold) was much higher than that in excitation (1.2-fold). Particularly impressive was a large shift in the locations of inhibitory synapses. In control animals, 16% of inhibitory synapses were on spines, while in the stimulated animal, this number increased to 28%, consistent with most new inhibitory synapses occurring on spines (Figure 1).

To examine the stability of these new synapses, Knott et al. performed serial EM analysis on animals that had

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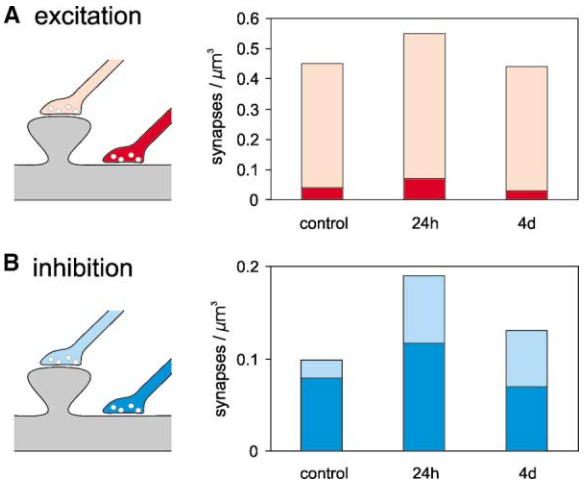

Figure 1. 24 hr of Whisker Stimulation Resulted in a Transient Increase in Synapse Density in the Barrel Cortex and a Shift of Inhibitory Synapses from Shafts to Spines

(A) Total excitatory synapse density increased after 24 hr whisker stimulation (24 h) but returned to normal after 4 days (4d). Most excitatory synapses occur on spines (pink), with a small proportion on dendrites (red).

(B) There was a similar absolute increase in total synapse density of inhibitory synapses (24h), but because there are fewer inhibitory synapses overall, the result is a larger proportional increase in inhibition. Total inhibition remains somewhat elevated after 4 days (4d), although not significantly. Most inhibitory synapses occur on dendrites (dark blue), with a small proportion on spines (light blue). Following whisker stimulation, there is a shift in the distribution of inhibitory synapses onto spines (24hr, 4d).

one whisker stimulated for 24 hr and were subsequently returned to their home cages. After 4 days, spine density and total synapse density had returned to prestimulus values, demonstrating that the experience-dependent increase in synapse density was transient. However, the distribution of inhibitory synapses remained shifted toward spines, with 32% of inhibitory synapses sharing spines with excitatory synapses (Figure 1). This suggests that some of the newly formed inhibitory synapses persisted for at least several days.

What could be the functional significance of inhibitory synapses on spines? In terms of inhibition, synapses on spines and dendrites with similar conductances will have essentially equal effects on neuronal excitation (Svoboda et al., 1996). However, excitatory and inhibitory synapses sharing the same spine will be spatially in close proximity and therefore poised to interact chemically. For example, when inhibitory neurons fire bursts of action potentials, GABA escapes the synaptic cleft to activate metabotropic $GABA_B$ receptors (Isaacson et al., 1993). Since activation of $GABA_B$ receptors on presynaptic terminals of excitatory neurons depresses neurotransmitter release, spillover of GABA from inhibitory synapses onto excitatory terminals could provide additional functional inhibition of cortical circuits (Isaacson et al., 1993).

Homeostasis versus Hebbian Mechanisms

A dominant idea in synaptic plasticity is that refinement of synaptic connections should obey correlation-based rules, such as those proposed by Hebb. However, it is sometimes not appreciated that these mechanisms can only work in conjunction with powerful homeostatic mechanisms that keep neuronal activity in a reasonable operating range, preserving network stability (Turrigiano and Nelson, 2000). Homeostatic rules predict that increases in network activity, such as those produced by the chronic whisker stimuli used in the Knott et al. study, should lead to a decrease in excitability. The synaptic changes observed by Knott et al. are consistent with homeostatic mechanisms working to reduce the excitation flowing into cortex from the overstimulated whisker. The observed increase in inhibition will act to reduce the excitation of layer 4 neurons in response to sensory stimuli.

This picture is consistent with the physiological consequences of the 24 hr whisker stimulation protocol. Knott et al. performed single unit recordings in layer 4. Recording from neurons in the stimulated barrel, they observed a decrease in sensory stimulation evoked responses. Interestingly, the decrease was not observed in the early onset phase of the response, but in later poststimulus epochs (12–25 ms and 50–100 ms), and was no longer present after 4 days. The decrease in response in the 12–25 ms epoch may be due to an increase in feed-forward inhibition, presumably mediated by newly formed inhibitory synapses on spines. Comparing measurements from stimulated and unstimulated barrels would make these eletrophysiological measurements more easily interpretable.

Why then is there an increase in excitatory synapses after whisker stimulation? A simple hypothesis can be formulated based on absolute numbers. After stimulation, the increase in the number of excitatory synapses exactly matches the number of additional inhibitory synapses (Figure 1). The increase in inhibitory synapses can be accounted for by new synapses on spines. Furthermore, spines with an inhibitory synapse always also contain an excitatory synapse (Micheva and Beaulieu, 1995). Together these data suggest that, in response to whisker stimulation, inhibitory synapses grow preferentially on new spines and that spine growth or stability requires the existence of an excitatory synapse.

Similar approaches combining ultrastructural analysis with single unit measurements could be used to address some of the important remaining questions. Do inhibitory and excitatory synapses grow simultaneously or in a stereotyped sequence? What aspects of the stimulus determine the number and types of synapses that are generated? Do receptive field changes correlate with growth of new synapses? And do new synapses form in response to behaviorally meaningful stimuli?

Molecular Mechanisms of Activity-Dependent Synaptogenesis

How does increased activity lead to synaptogenesis? Synaptic structural plasticity is thought to rely upon gene expression and protein translation. In adult animals, whisker stimulation causes upregulation of immediate-early genes (Melzer and Steiner, 1997), and experience-dependent plasticity paradigms cause CRE-mediated gene expression (Barth et al., 1999). One particularly attractive candidate CRE-dependent gene is BDNF.

BDNF has roles in dendritic morphogenesis (McAllister et al., 1996) and modification of both excitatory and inhibitory synapses (Rutherford et al., 1998). BDNF is proposed to have a critical role in development of inhibition that defines the critical period for cortical map plasticity (Huang et al., 1999). Finally, it has been demonstrated that 6 hr of whisker stimulation causes an increase in BDNF expression specifically in the stimulated barrel cortex (Rocamora et al., 1996).

These data support a model in which activity-dependent upregulation of BDNF leads to spine growth and recruitment of new inhibitory synaptic connections. However, it remains to be demonstrated that transcription or translation are required for activity-dependent synaptogenesis. Examination of animals in which CRE-dependent signaling or BDNF synthesis is perturbed would constitute an important test of the model. Of course, it is naive to assume that the observed changes result from the action of a single gene product. Physiological examination of mouse knockouts has already demonstrated roles for CamKII and CREB in aspects of barrel cortex plasticity (Fox, 2002). Genetic manipulations will be critical to determine roles of candidate molecules in experience-dependent synapse formation and should be more compelling and informative once under precise temporal and spatial control.

Experience-Dependent Plasticity of Dendritic Spines In Vivo

The Knott et al. paper provides evidence for rapid, activity-induced synaptogenesis in the adult cortex and suggests many avenues for further investigation. For example, where and between which cell types do the new synapses form? Are new inhibitory synapses formed on existing spines, or do spines grow to make inhibitory connections?

Addressing these types of questions will demand time-lapse observations of synaptic structure in vivo. Recent advances in imaging technology have permitted imaging of GFP-labeled neurons in vivo. Such measurements are powerful because they permit real-time observation of dendritic spines, and therefore they can detect dynamic structural changes even under conditions where spine densities remain constant on average. Lendvai et al. imaged layer 2/3 pyramidal neurons infected with Sindbis-EGFP in the developing barrel cortex of the living rat (Lendvai et al., 2000). Sensory deprivation by unilateral whisker trimming for 2 days caused a 40% reduction in the protrusive motility of dendritic spines in deprived regions and degradation in the tuning of layer 2/3 receptive fields. The authors conclude that sensory experience drives structural plasticity in dendrites, which may underlie reorganization of neural circuits during plasticity.

An important step will be to perform similar studies in adult animals. What is the baseline structural plasticity in the normally behaving adult animal? Over what time scales do dendrites in the adult brain change? What are the consequences of manipulations of sensory experience? In the adult animal, such experiments will require the development of chronic imaging at the level of single synapses. But imaging synaptic structure is limited in that it can never with certainty capture synaptogenesis. In the future, the use of fluorescent proteins that report Ca^{2+} concentration may help by allowing the simultaneous imaging of synaptic structure and synaptic function. However, in the near-term, correlating serial section EM with in vivo imaging will be more realistic. In this way, it should be possible to identify where new synapse addition occurs and precisely which synapses are being added. It is through in vivo imaging in combination with ultrastructural analysis that we will have our first convincing glimpses of nascent central synapses in the intact adult brain.

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